

OXIDATION OF MONOSACCHAROSES AND THEIR MONOMETHYL
DERIVATIVES TO DICARBOXYLIC ACIDS

by

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INTRODUCTION

Carbohydrates have always played an important role in the diet of animals, in the prehistoric formation of coal and petroleum, in the formation of shelter and clothing for animals, and more recently, in the preparation of many useful materials such as lacquers and synthetic fibers.

Early scientific investigation dealing with the structure of carbohydrates began about 1885 when Kiliani (4) undertook to determine the position of the carbonyl group in some of the monosaccharoses. Two years later, Emil Fischer (2) and his pupils began classical investigations in an attempt to learn more about the structure of these closely related compounds. Since these early investigations this phase of carbohydrate chemistry has received the attention of many illustrious chemists. Results of this continued study have provided fundamental information of the close structural relationship of the various monosaccharoses. In some phases of this work, however, detailed procedures used for determining these relationships are still lacking.

The object of this investigation was to develop a satisfactory method for preparing polyhydroxy dicarboxylic acids from the corresponding sugars and their monomethyl derivatives. Methods of preparing the monomethyl derivatives of monosaccharoses were also studied. The investigations were limited to the oxidation of D-glucose, L-xylose and α -monomethyl D-glucoside and the methylation of D-glucose. Mild oxidation of monosaccharoses gives the corresponding monocarboxylic acids. Stronger oxidation yields the polyhydroxy dicarboxylic acids. From a study of the optical activity of many of the monosaccharoses and the dicarboxylic acids formed by their oxidation, the relative stereo or space arrangement of these molecules can be determined.

REVIEW OF LITERATURE

Accurate knowledge of the chemistry of carbohydrates actually started a little more than a century ago when glucose was shown to have the molecular formula $C_6H_{12}O_6$. During the next 50 years, three more hexoses, galactose, fructose, and sorbose, were discovered and found to have

the same molecular composition.

According to Cohen (30), hexoses were synthesized as early as 1861 when Butlerow found that by the addition of lime-water to a hot solution of trioxymethylene (a solid substance produced by the polymerisation of formaldehyde) methylenitan is formed. This product is described as a sweet yellow syrup, giving the ordinary reactions for sugar, but optically inactive and incapable of fermentation. Cohen (30) also states that Loew, in 1886, discovered that formaldehyde and lime-water, at ordinary temperatures, yield a sweet syrup of the formula $C_6H_{12}O_6$ which he termed formose; but this was also unfermentable.

Numerous investigations were undertaken to determine why these sugars differed in physical and chemical properties, although they all had the formula $C_6H_{12}O_6$. In 1887, Fischer (2, 3) succeeded in preparing α -acrose synthetically by treating glycerose with dilute alkali. α -Arose was found to be an equal molecular mixture of D-fructose and L-fructose.

Kilian (4, 5, 6), in 1885 and 1886, determined the position of the carbonyl group in L-fructose, D-glucose and D-galactose.

Ruff (10), in 1898 and Wohl (11, 12), in 1893 and 1897, worked out methods by which a higher sugar could be

converted to a lower sugar.

The early investigations on oxidation of monosaccharoses to their corresponding dicarboxylic acids were studied extensively by Sohst and Tollens (15).

Fischer (16) worked out a satisfactory method for preparation of potassium hydrogen saccharate. However, there is no complete method for the preparation of saccharic acid.

Rehorst (17) completed some important work on the study of saccharic acid in 1928. He was the first to isolate the pure, free saccharic acid in crystalline form. Other investigators obtained only the dilactone form of the acid.

The dilactone form of saccharic acid has a specific rotation of $+37.9^{\circ}$ and melts at $130^{\circ} - 132^{\circ}$ C., but the free saccharic acid melts at $125^{\circ} - 126^{\circ}$ C. and has a specific rotation of $+6.3^{\circ}$. It is easy, therefore, to distinguish between the dilactone form and the free acid by the specific rotation.

Evans (21, 22) and Evans, et al. (23, 24, 25, 26) have recently carried on investigations on the oxidation of carbohydrates in neutral and weakly alkaline solutions. His work resulted in breaking down the molecules into sim-

ple products and from the study of the various products obtained, he showed certain characteristic linkages within the sugar molecules.

Fischer (16) has worked out a good method by which the α -monomethyl glucoside can be easily prepared. This method, however, puts the methoxy group in the α - and not the β -position.

Whitnah and Milberry (20), Schluback (19) and others have recently succeeded in methylating sugars with dimethyl sulphate which places the first methoxy group in the β -position. Dimethyl sulphate will, however, continue to methylate the hydroxy groups as far as desired but methyl alcohol methylates only the aldehyde end.

EXPERIMENTAL

Oxidation of D-Glucose

The oxidation was carried out as outlined by Fischer (16). One hundred grams of anhydrous DGlucose were heated in a large evaporating dish on a steam bath with 609 cc. of dilute nitric acid (sp. gr. 1.15). The mixture was stirred continuously by means of a mechanical stirrer until concentrated to a syrup. This syrup was dissolved in a little

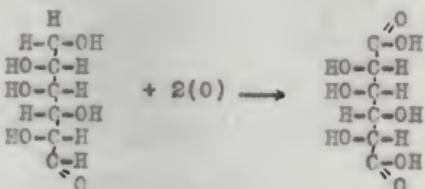
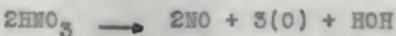
water and again evaporated until the mixture began to acquire a slight brown color. Heating was then discontinued.

Various concentrations of nitric acid were used to oxidize D-glucose to determine which would give the best yield of saccharic acid. There was not much variation in the yield as long as the nitric acid was approximately the same concentration as that specified by Fischer (16) but the yield was slightly increased by adding a little more nitric acid before the mixture was evaporated to a syrup. Too high or too low concentration of acid decreased the yield.

If the heating and stirring were discontinued before a syrup was obtained and the mixture allowed to stand over night, oxalic acid always crystallized out. These crystals, when washed and recrystallized from hot water, always melted sharply at 100° C.

It was found unnecessary to use anhydrous D-glucose to obtain a good yield of D-saccharic acid if the water of hydration of the sugar was taken into consideration and correspondingly less acid used.

The reactions are represented by the following equations:



D-glucose

D-saccharic acid

Formation and Separation of Potassium Hydrogen D-Saccharate

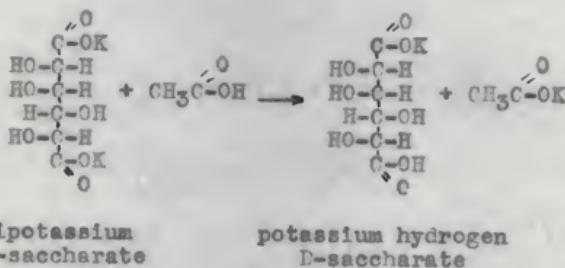
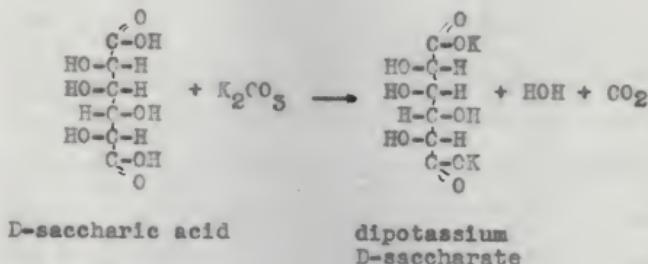
The slightly brown syrup of D-saccharic acid, which was mentioned previously, was dissolved in about 300 cc. of water and neutralized with a concentrated solution of potassium carbonate. An excess (about 50 cc.) of dilute (50%) acetic acid was then added. The solution was continually stirred as the potassium carbonate and the acetic acid were added. Heat from a steam or hot water bath was again applied and stirring continued until the mixture was evaporated to a small volume (about 160 cc.). This solution was then transferred to a beaker and occasionally stirred as it was cooled. When the crystals of potassium hydrogen D-saccharate began to form the mixture was placed

in the cold for several days and stirred frequently. The precipitate of potassium hydrogen D-saccharate which formed was filtered off with suction and washed with a little cold water. It was transferred to a beaker and dissolved in a minimum quantity of hot water and Norite A was added to adsorb colored impurities. The Norite was filtered off with suction through a layer of asbestos. Upon cooling the filtrate, potassium hydrogen D-saccharate crystallized out. Occasionally the product obtained was not colorless, making recrystallization from hot water necessary.

It was observed that a better yield was obtained when the mixture containing the potassium hydrogen D-saccharate was evaporated to a smaller volume than that specified by Fischer (16).

Frequent stirring as the salt crystallized out prevented caking and made the product much more easily washed and purified.

The reactions are represented by the following equations:



The potassium hydrogen D-saccharate was identified by its neutral equivalent which was found to be 264 and the calculated neutral equivalent was 267.

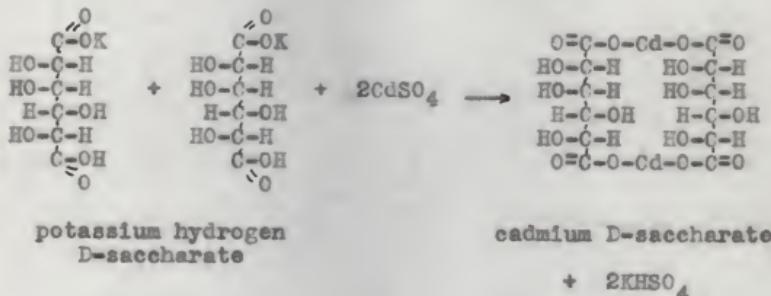
Preparation of Cadmium D-Saccharate

A hot saturated solution of potassium hydrogen D-saccharate, containing an excess of the undissolved salt, was

heated on a steam or hot water bath and stirred with a mechanical stirrer. As the solution was heated and stirred, the calculated amount of a hot saturated cadmium sulphate solution was added. After about ten minutes, the heat was removed but stirring was continued for three or four hours. During this time slightly soluble cadmium D-saccharate was formed and slowly separated from solution. It was allowed to settle and the supernatant liquid was decanted through a Buchner funnel. The precipitate was washed several times by decantation with a small amount of cold water, then transferred to the Buchner funnel and again washed.

The cadmium D-saccharate is insoluble in hot water, therefore, it cannot be further purified by crystallization. The yield was about 45 - 55 per cent of the theoretical. If the reaction mixture were allowed to stand without stirring for two weeks, only a 40 per cent yield could be obtained. It was found that a better yield was obtained when the reaction mixture was stirred for several hours. When the mixture was allowed to stand without stirring, the reaction was also much slower.

The reactions are represented by the following equations:



The cadmium was identified as cadmium D-saccharate by determining the per cent cadmium present in the salt. The method of analysis consisted of digesting the cadmium D-saccharate in a very small amount of concentrated nitric acid until solution occurred and then diluting. The dilute acidic solution was saturated with hydrogen sulphide for several hours to precipitate the cadmium as cadmium sulphide. Then the mixture was boiled to drive off most of the excess hydrogen sulphide and the remaining excess was destroyed with dilute iodine solution. Starch solution was used as an indicator. The mixture was made very dilute, then made strongly acid with concentrated hydrochloric acid and titrated with a standard iodine solution. The analysis showed 42.51 per cent of cadmium in the cadmium D-saccharate compared with the calculated value of 43.12 per cent.

Attempts were made to prepare sodium hydrogen D-saccharate by using sodium carbonate in place of potassium carbonate. The product was so soluble in cold water that it could not be successfully recrystallized. Solutions of these crystals showed no neutral equivalent. It was concluded therefore that the neutral salt and not the acid salt had been obtained. However, if the crude product was treated with a solution of cadmium sulphate, in the same manner used with the potassium hydrogen D-saccharate, a good yield of cadmium D-saccharate was obtained.

Separation of D-Saccharic Acid

Seventy-five grams of cadmium D-saccharate was transferred to a two-liter three-neck pyrex flask equipped with a device (figure 1) for stirring under a slight pressure. After adding one liter of water, the mixture was stirred by means of this device to insure suspension of the cadmium salt. The suspension was kept saturated with hydrogen sulphide under slight pressure for about 14 hours.

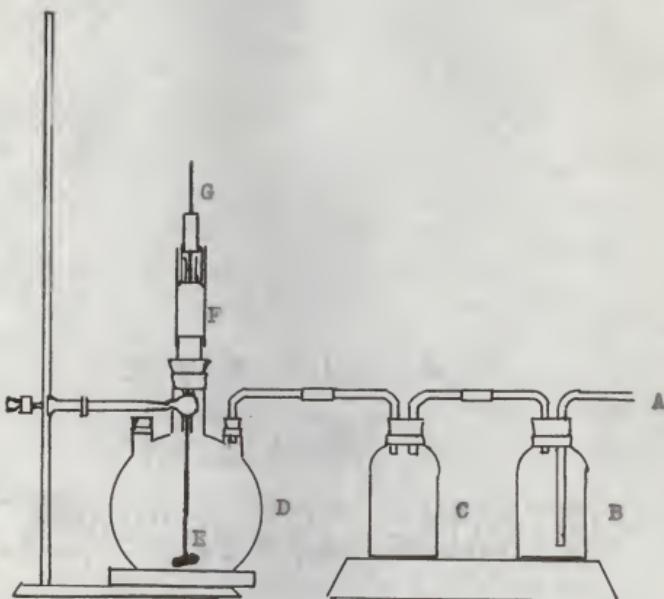
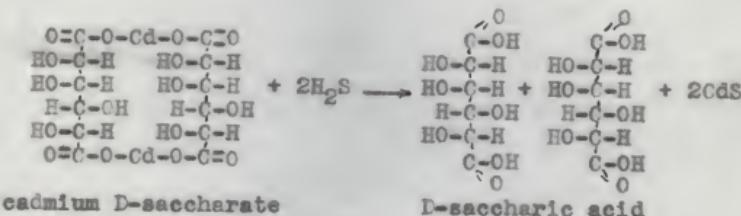


Figure 1. Illustration of the device used for stirring under slight pressure.

- A - tube leading to hydrogen sulfide generator
- B - bottle containing water
- C - safety bottle
- D - three-neck pyrex flask
- E - pyrex glass paddle for stirring
- F - mercury seal
- G - pyrex rod leading from paddle to electric motor

The cadmium was precipitated as cadmium sulphide leaving D-saccharic acid in solution. The cadmium sulphide was filtered off through a layer of asbestos with suction and the solution evaporated to dryness under reduced pressure.

The reactions are represented by the following equations:



The solution was evaporated at about 60° C. on a water bath with the bath kept below 70° C. to prevent decomposition and darkening in color. The colorless residue was taken up in a mixture of 400 cc. of absolute ethyl alcohol and 800 cc. of isobutyl alcohol (17). The mixture was concentrated to a small volume by again evaporating under reduced pressure at about 60° C. The alcohol solution of the acid was transferred to a beaker and placed in the ice box to crystallize. After one week, the crystals of

D-saccharic acid began to form and continued to grow.

Rehorst (17) has given a method by which these crystals may be further purified by recrystallizing them from ethyl alcohol and then drying in a vacuum desiccator over sulphuric acid.

Ethyl alcohol, diethyl ether, acetone, iso-propyl alcohol, iso-butyl alcohol, and various mixtures of the same were also used in attempts to crystallize D-saccharic acid. Among these, however, the only crystallizing solvent found to be at all satisfactory was a mixture of 50 per cent ethyl alcohol and 50 per cent n-propyl alcohol which yielded the dilactone form.

The dilactone form of the acid was identified by its neutral equivalent and its specific rotation. The results of the analyses were as follows:

	in literature	observed
Specific rotation	+37.9°	+35.2°
Neutral equivalent	210	216

Methylation of D-Glucose

Attempts to prepare monomethyl glucoside by means of dimethyl sulphate were unsuccessful. The method which was

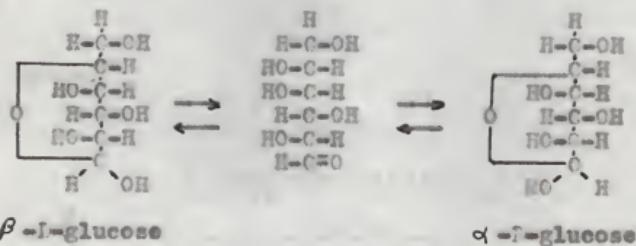
used is as follows (19): Thirty-six grams (.2 mol.) of anhydrous D-glucose were dissolved in 200 cc. of water. Thirty cc. (.3 mol.) of dimethyl sulphate were added, drop by drop, from a separatory funnel. The solution was kept slightly alkaline by allowing a 30 per cent solution of sodium hydroxide to flow dropwise into the reaction mixture. Dibromothymosulphonphthalein (brom thymol blue) was used as an outside indicator and the solution was treated for alkalinity every five minutes. After one week, during which work was discontinued at night, all the dimethyl sulphate had been added. The solution was then warmed to 40° C. and carefully neutralized with dilute sulphuric acid. The calculated amount of barium carbonate was added to precipitate the sulphate ions. The barium sulphate formed was filtered off and the filtrate evaporated to a syrup under reduced pressure. This syrup was taken up in 60 cc. of pyridine, mixed with 60 cc. of acetic anhydride and shaken in a mechanical shaker for 24 hours. The reaction mixture was then poured into two liters of ice water. A white, somewhat sticky product separated out. This was supposed to be the tetracetyl derivative of the β -monomethyl D-glucoside. Recrystallizing this material several times from hot water gave long needle shaped crystals hav-

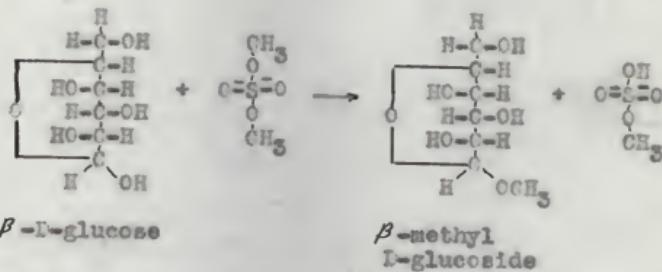
ing a melting point of 130 - 131° C. These physical properties were evidence that the crystalline product was β -D-glucose pentacetate.

No record of the properties of the tetracetate derivative of β -methyl D-glucoside could be found in the literature, consequently the method of Bernstein (27, 28, 29) was used to determine whether methoxy groups were present or not. Several determinations by this method were made but no methoxy group was found to be present.

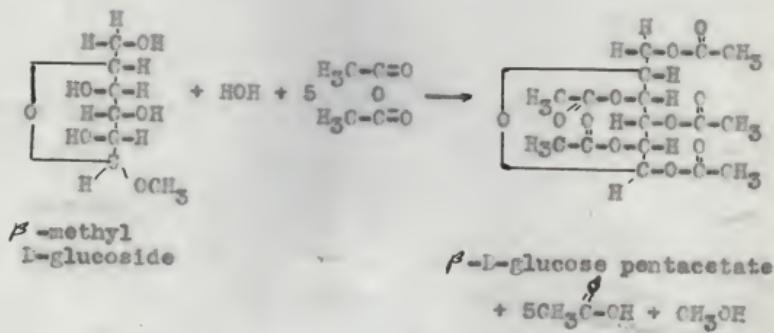
It was concluded from these results that the product was β -D-glucose pentacetate.

The reactions are represented by the following equations:





Reactions represented by the following equations must have taken place:



Since the methylation with dimethyl sulphate was unsuccessful, D-glucose was methylated with methyl alcohol in the presence of dry hydrogen chloride according to the method of Fischer (16).

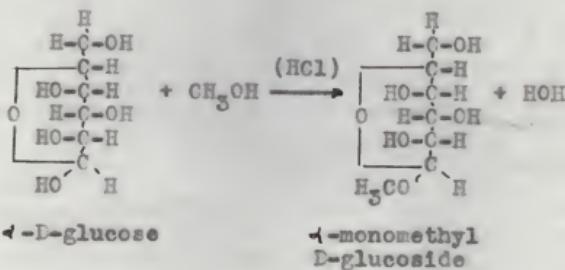
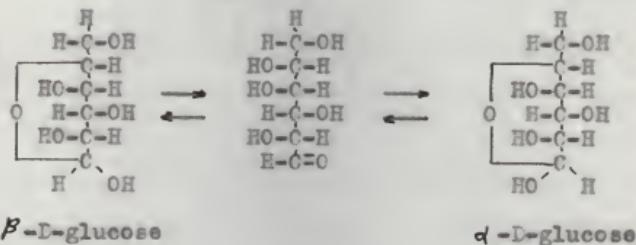
Dry hydrogen chloride was passed into 300 cc. of ab-

solute methyl alcohol until a solution of about .6 per cent hydrogen chloride was obtained. This mixture was titrated with .1 normal sodium hydroxide to find the dilution necessary to obtain a .25 per cent hydrogen chloride solution.

To 200 grams of the .25 per cent hydrogen chloride solution, 50 grams of anhydrous D-glucose were added and boiled under a condenser set upright for refluxing until the glucose was dissolved. The solution was introduced into pyrex glass tubes. The tubes were sealed and introduced into iron pipes which were placed in a water bath on a hot plate and boiled for 50 or 60 hours. Occasionally one of the tubes would blow up but the iron pipes protected the other tubes and surroundings.

After 50 or 60 hours of heating, the tubes were removed; the liquid was transferred to a beaker and evaporated to about one-third of its original volume. α -methyl D-glucoside began to crystallize in small needle-shaped crystals. After standing for about 24 hours, the crystals were filtered off with suction. They were purified by recrystallization from hot ethyl alcohol. About a 45 per cent yield was obtained. The product was identified as α -monomethyl D-glucoside by its melting point (165° C.) and by the shape of the crystals.

The reactions are represented by the following equations:

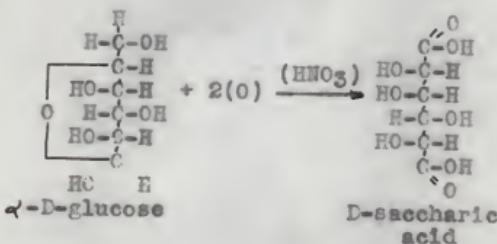
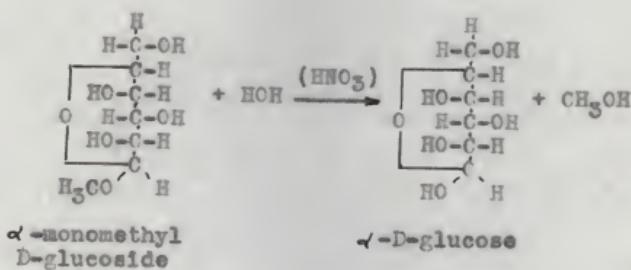


Oxidation of α -Monomethyl D-Glucoside

Forty grams of α -monomethyl D-glucoside were oxidized by the method of Fischer (16). Approximately the same yield of potassium hydrogen D-saccharate was obtained as when D-glucose was oxidized. It was concluded therefore that the methoxy group hydrolyzes off rapidly and leaves

the free sugar groups open for oxidation before the oxidizing agent can exert an appreciable effect upon the primary alcohol group at the other end of the molecule.

The reactions are represented by the following equations:



Oxidation of L-Xylose

Attempts to prepare the potassium hydrogen salt of

the trihydroxy glutaric acid, corresponding to L-xylose, were unsuccessful. Using the same method by which potassium hydrogen D-saccharate was prepared from D-glucose gave a neutral product which was very soluble in cold water and therefore difficult to crystallize. This product had no neutral equivalent which is some evidence it was the dipotassium salt. It was concluded that this method of obtaining the trihydroxy glutaric acid, corresponding to L-xylose, could not be used efficiently. Other methods of oxidation were not attempted.

SUMMARY AND CONCLUSIONS

D-saccharic acid was prepared by oxidizing D-glucose with nitric acid. The D-saccharic acid was changed to the dipotassium D-saccharate with potassium carbonate, then treated with acetic acid to obtain potassium hydrogen D-saccharate, the form in which it was isolated. The potassium hydrogen D-saccharate was then treated with cadmium sulphate solution to obtain cadmium D-saccharate which was then changed to the D-saccharic acid by treating with hydrogen sulphide. D-saccharic acid was carefully crystallized from a mixture of ethyl alcohol and isobutyl alcohol to obtain the free acid. The dilactone form was

crystallized from a hot mixture of 50 per cent ethyl alcohol and 50 per cent N-propyl alcohol.

Glucose was easily methylated with methyl alcohol in the presence of dry hydrogen chloride to obtain α -monomethyl glucoside.

This substance was as easily oxidized and gives the same yield of D-saccharic acid as does D-glucose.

L-xylose cannot be successfully oxidized to the corresponding tri-hydroxy glutaric acid in the same manner as D-saccharic acid was obtained from D-glucose.

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